STATUS OF BEHAVIORAL AND PHYSIOLOGICAL "RESISTANCE"*

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THE study of acquired humoral resistance to infectious agents has received just attention in the past 50 years. The antigen-antibody concept has dominated a considerable portion of our thoughts and experiments on resistance of animals and has reached a high state of mutation in the field of immunochemistry. We have tended to neglect detailed analyses of many phenomena of host-parasite incompatibility which have their origins in certain other qualities of hosts and parasites.

Dubos (1954) has pointed out that present-day literature usually describes disease as a conflict, one party being an "invader" and the other using "defense mechanisms." We are accustomed to thinking in terms of the resistance or susceptibility of the host to the parasite. However, the host and the parasite may be regarded as an entity, and we may think in terms of those factors which act to prevent the establishment of this entity. In these terms, resistance and susceptibility will obviously involve attributes of the parasite or the host, or, as is more often the case, both, and would embrace that perennially favored subject for speculation, host specificity.

Schneider (1951) argued cogently that we should distinguish sharply between resistance and susceptibility. These terms were operationally defined from experience with nutritional factors: those factors in the nutrition which when withheld from the host decrease the extent or effect of an infection and when supplied increase the effect were termed "susceptibility factors." Those nutritional factors which when withheld increase the extent of infection, and when supplied decrease the effect, were termed "resistance factors." I agree with Schneider's suggestion that we are dealing with separable phenomena and his choice of terms seems reasonable. However, the definitions, as presented by Schneider, are useful only in considering the relation of nutrition to disease. It seems to me that we may conveniently deal with resistance and susceptibility as separate concepts in more general terms. What we are truly dealing with in

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the case of susceptibility are those aspects of the environment furnished by the host which are not directly dependent on previous or present occupation by the parasite. In other words, susceptibility is a physiological state of the host in which the parasite is supplied with its life needs and insusceptibility is the state in which these life needs are not satisfied, neither state involving a host response to the parasite. There would obviously be intergrading conditions.

Resistance may be defined as those alterations of the physiological state of the host which represent a response to previous or present experience with the parasite or a chemically related entity. In this category antibody production would, of course, be included. Clearly in an individual host we may have both susceptibility and resistance. By these definitions we may have a highly susceptible host which is also a highly resistant one.

In preparing the following discussion, an attempt has been made to discard resistance, as defined above, from consideration. At the same time, some attention has been given to some partners in parasitism, ordinarily falling in the jurisdiction of plant pathology. The latter course of action seemed desirable because of what the present writer considers to be an unjustifiably complete separateness of students of plant and animal disease phenomena. The disadvantages of this separateness are readily seen in the history of the sciences of infectious disease. Prévost (1807) demonstrated conclusively that wheat bunt is an infectious disease and that the etiological agent is a microorganism. He devised a chemotherapy for the disease and distinguished between fungicidal and fungistatic effects. Comparable work with animals was not forthcoming for almost 40 years. It may be significant that Pasteur's studies on infectious diseases in animals developed from his studies on the "diseases" of non-zoological materials, beer and wine.

In this paper I shall attempt to present evidence to support the general concept that insusceptibilities to the attainment and maintenance of parasitisms are widely distributed and probably common in nature. The pretense cannot be made that this is a complete review of the examples available in the literature. It may also be added that with a moderate effort many other examples can probably be discovered in nature.

Internal Factors Influencing Parasitisms

It seems obvious that for the establishment of parasitism, the environmental characteristics in or on a host must be compatible with the life requirements of the parasite. More than this, if the initial events of establishment involve multiple physical or chemical stimuli leading to the alteration of behavioral or other physiological characteristics of the

parasite, it may be expected that the duration and sequence of these environmental stimuli may be important in promoting or preventing establishment of parasitism. What is the evidence that specific chemical substances, produced by the host and not representing a response to the parasite, interfere with maintenance of parasitism? A number of examples are available.

No discussion of this subject should overlook the studies of Dr. Ackert and his collaborators on the anti-*Ascaridia* substance in duodenal mucus (see Frick and Ackert, 1948 and earlier papers by Ackert, *et al.*). It was shown that, with ageing of the chicken, there is an increased production of a substance associated with duodenal mucus which inhibits the establishment and growth of *Ascaridia* in the host or growth of the worm *in vitro*. This substance was found to be soluble in 0.8 percent saline and heat stable. Because of the latter property, it was concluded that the substance is not an antibody. Eisenbrandt and Ackert (1941) showed that mucus from dogs and swine had a lethal effect on *Ascaridia in vitro* and suggested this may have bearing on the host specificity of this nematode. Further studies on the nematode-inhibiting substances associated with mucus have not been forthcoming. It should be feasible to characterize the material with greater precision.

In collaboration with Dr. Lee Douglas and Mr. John Simmons, I have recently had an opportunity to study the osmotic properties of certain tapeworms of elasmobranch fishes. Incubation of these tetraphyllideans in salt solutions showed that the worms change in wet weight as a function of the salt content of the medium and that chloride is gained or lost, depending on the concentration in the medium. It was shown that the addition of urea to salt solutions prevents an initial imbibition of water by the worms in all salt solutions used (Figure I). It was found that the blood and the intestinal contents of the hosts and of freshly collected worms have a very high urea content. In *Calliobothrium verticillatum*, almost 4% of the dry weight of the worm consists of urea. Urea is lost from tetraphyllideans when they are incubated in urea-free media and the concentration in the tissues of the worm is a function of the concentration of urea in the suspending medium. Further, it was found that shifts in urea content of worms in artificial media are accompanied by chloride shifts. There is an inverse relationship of tissue chloride to concentration of urea in the medium. This supports an hypothesis that urea plays a role, perhaps an indirect one, in maintaining ionic balance in these animals and may be extremely important in determining their host distribution. During these studies it was also observed that urea, at concentrations below those of the elasmobranch gut fluids, is highly toxic to several species of tapeworms from birds, mammals, and fishes. The toxic

effects were found to be irreversible. In addition, six species of trematodes from teleost fishes were rapidly paralyzed in salt solutions containing urea. The data support the conclusion that the high concentration of urea in the elasmobranch gut probably serves as an effective barrier to infection of these hosts by digenetic trematodes or cyclophyllidean and tetrabothriid cestodes (Read *et al.*, 1958).

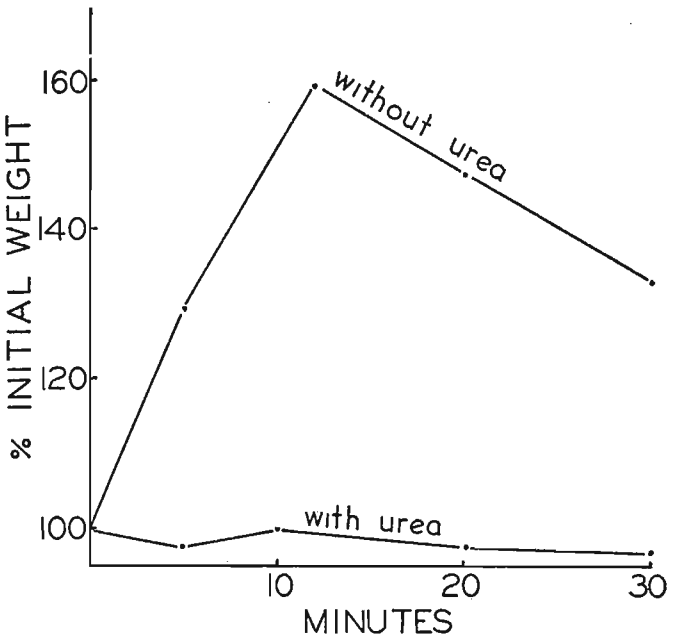

FIG. 1. Changes in the wet weight of *Calliobothrium verticillatum* incubated in solutions with or without urea. The incubations were carried out at room temperature. The solutions contained the following per liter: 200 mM NaCl, 4.4 mM KCl, 5.1 mM $CaCl_2$, and 2.9 mM $MgCl_2$. Urea was added to a concentration of 200 mM per liter.

In a series of studies on the development of pseudophyllidean tapeworms Michajlow has reported that many species of copepods will feed on coracidia. This work has been summarized by Michajlow (1951). It was shown that the fate of such coracidia in the various copepods differed, depending on the action of the digestive fluids. In some copepods, onchospheres of a given pseudophyllidean were killed by the digestive juices. These species of copepods obviously cannot serve as intermediate hosts. In other copepods, most of the onchospheres of a given tapeworm are killed by the digestive juices; other things being equal, such species would serve as what Michajlow called auxiliary hosts. A third group of

copepods secretes digestive juice which seems to have no deleterious effects on the onchospheres. Representatives of the third copepod group may be expected to serve as the usual intermediate hosts. Michajlow's observations are an interesting example of apparent chemical barriers to infection and would merit additional study and confirmation.

Among plant parasites, the classical example of chemical exclusion of a parasite by a potential host is seen in the insusceptibility of some onions to the smudge organism, *Colletotrichum circinans*. This fungus begins its relationship with a host by a saprophytic development on the outer nonliving scale of the plant. Eventually it penetrates the underlying living scales and produces pathology. Onion varieties in which the bulb scales are pigmented are highly insusceptible to smudge. Associated with the colored flavone compounds in the dry outer scales are colorless water-soluble phenols, catechol and protocatechuic acid (Link and Walker, 1933). These are toxic to the smudge organism, preventing germination and penetration. That this protection is afforded by the dry outer scales is shown by the fact that if the outer scales are removed and the underlying fleshy scales are exposed to the fungus in a moist chamber, parasitism ensues (Walker, 1923).

Another instance of insusceptibility due to the elaboration of phenolic compounds has been reported by Schaal and Johnson (1955). These authors showed that potatoes which were insusceptible to parasitism by the scab organism, *Streptomyces scabies,* contain high concentrations of chlorogenic acid. Evidence was adduced that when chlorogenic acid is oxidized by tyrosinase the resultant quinones are toxic to the scab organism. Russet color in potatoes is genetically linked to scab-insusceptibility, presumably because the color is due to the presence of the colored quinones.

Beck (1957) studied the role of growth-inhibiting chemical factors in the insusceptibility of corn to the establishment and survival of the European corn borer, *Pyrausta nubilalis*. The total quantity of growth-inhibiting substances in the tissues of various inbred corn strains was determined by bioassay, using corn borers maintained on purified diets. The total inhibitor activity, as determined by bioassay, was found to be in agreement with susceptibility ratings of the particular strains as determined in field tests. It was shown that three antiparasitic chemical entities are involved: Factor A (6-methoxy-benzoxazolinone) was found to be important in the insusceptibility of early plant growth stages, but negligible after the development of the visible corn tassel. The chemically-undefined Factors B and C contributed about equally to insusceptibility of the internode, leaf sheath, husks, and silk tissues. Virtanen and Hietala (1955) reported that insusceptibility to the fungus, *Fusarium,* in rye could be

attributed to the presence of a fungus-inhibiting substance related to Factor A, 2(3)-Benzoxazolinone, in the seedlings. Virtanen, *et al.* (1956) showed that fungus-insusceptible corn contained Factor A.

It may be noted that some of Beck's borer-insusceptible corn strains were originally selected for insusceptibility to stalk-rot, a fungus disease, and were subsequently found to be borer insusceptible. Johann and Dickson (1945) had described the presence in corn of an inhibitor of *Diplodia,* the stalk-rot organism. The inhibitor is apparently identical with Factors A or C involved in insusceptibility to the corn borer. This is of great interest since what might first appear to be genetic linkage of parasite-inhibiting factors resolved into a common causal basis of insusceptibility to parasites of different kingdoms.

A subtle form of insusceptibility is suggested by recent work on the effects of bile salts on the metabolism of the strobilae of certain tapeworms (Rothman and Read, 1957; Rothman, in press). In the presence of physiological concentrations of bile salts, the metabolism of *Hymenolepis* and *Oochoristica* was inhibited to a much greater extent at pH 7.0 than at pH 7.4. If this is considered in terms of the complications of intestinal physiology, it is readily seen that effective inhibition by bile salts may occur in some hosts and not in others. Although they have been reviewed elsewhere (Read, 1950), salient points may be indicated: (1) There is a linear hydrogen-ion gradient in the small intestine, the pH increasing from anterior to posterior. (2) There is a linear bile-salt-concentration gradient, the sites of absorption differing with the quality of bile salt. (3) The kinds of bile salts secreted are phylogenetically different. (4) The anatomical position of the intestinal orifice of the bile ducts shows considerable variation among different species and would be expected to condition the extent and nature of the gradients mentioned above. Thus it may be expected that, in some hosts, these variables will result in environments which are incompatible with survival of these worms.

There is evidence that the action of specific intestinal secretions may be of importance in the decystment and evagination of tapeworm larvae. Various workers have reported that bile or bile salts cause evagination of tapeworms. Malkani (1933) produced rapid evagination of cysticerci of *Taenia tenuicollis* and *T. saginata* by treating them with diluted bile *in vitro.* DeWaele (1934) made similar observations with *T. pisiformis.* Both Malkani and deWaele noted that the bile salts, glycocholate and taurocholate, had different effects on *Taenia* spp. Malkani noted that *T. tenuicollis* evaginated more slowly in glycocholate than in taurocholate. DeWaele reported that glycocholate is toxic for *T. pisiformis,* whereas the worm readily tolerates taurocholate. DeWaele postulated that this

effect explains the fact that *T. pisiformis* occurs only in hosts (dogs) having taurocholate and lacking glycocholate as a bile constituent.

In addition to these examples of substances which may interfere with parasitism by a directly deleterious effect on the parasite, there are cases in which the establishment of parasitism may not occur because of the disoperation of stimuli. When multiple stimuli are involved in the establishment of a parasitism the sequence and timing of stimuli may be important. It has been shown that intestinal emptying time may be a critical factor in determining the degree of establishment of *Hymenolepis nana* (Larsh, 1947) and *H. diminuta* (Read and Voge, 1954). When timing is compounded with other required factors, considerable specificity of effect may ensue. Read (1955) attempted a quantitative study of the factors involved in the excystment of the cysticercoid of *Hymenolepis diminuta.* It was postulated that a complex of five factors was involved. These were a priming effect of gastric juice, specifically pepsin at low pH, an excitatory effect of bile salts, a denaturation effect of bile salts on the proteins of the larval cyst, a proteolytic effect of trypsin, and an effect of elevation of temperature. It was shown that the final result, excystment, depended on the interactions of these factors. Rothman (1958) has extended these findings and has made a comparative study of the effects of intestinal secretion components on cysticercoids of *Hymenolepis diminuta, H. citelli, H. nana,* and *Oochoristica symmetrica,* and on the strobilocercus of *Taenia taeniaeformis.* He found rather marked quantitative differences in the rates at which the *Hymenolepis* spp. respond to these secretions. There was a characteristic maximum time of treatment in gastric juice beyond which further treatment had negligible effects; *H. diminuta* excysted most rapidly after a treatment of at least 30 minutes whereas *H. nana* responded maximally after 15 minutes' treatment. The cysticercoid of *H. nana* excysted about twice as rapidly in a solution containing trypsin and bile salts as *H. citelli* and *H. diminuta* if the worms had not been pretreated with artificial gastric juice. The rate of excystment of *H. citelli* larvae was slower in old larvae than in young ones; ageing was without effect on the rate of response of *H. diminuta* larvae. The excitatory response to bile salts appeared much more slowly in *H. citelli* than in *H. diminuta* cysticercoids. *Oochoristica* was found to be deleteriously affected by gastric juice and readily excysted in salt solutions containing bile salts at low concentrations. With *Oochoristica,* a definite optimum concentration of bile salts was observed; at concentrations above the optimum the worms evaginated but reinvaginated on continued exposure. *Taenia taeniaeformis* required treatment with pepsin before evagination would occur upon subsequent treatment in salt solutions which were neutral or slightly basic in reaction. Bile salts were not re-

quired for evagination of *T. taeniaeformis* and, at concentrations above 0.1%, bile salts caused a reinvagination of the strobilocercus. The known qualitative effects on tapeworm larvae of gastric juice, bile salts, and trypsin are summarized in Table 1. Rothman has reported a number of other quantitative differences between closely related species. Each species studied has been found to require interacting factors for excystment, and these differ considerably between species. There are, of course, a number of other untested variables which may have effects. However, the observations available support the hypothesis that the quality, quantity, and sequence of factors involved in the excystment of tapeworms may play an important role in determining whether a host is resistant to the initial establishment of a particular cestode species.

TABLE I

	Treatment in Gastric Juice	Effects of Bile Salts		Effect of Trypsin
		(Glycocholate)	(Taurocholate)	
Taenia				
pisiformis	0	(lethal)	++	0
tenuicollis	0	+	++	0
taeniaeformis	+	(0; reinvagination at high concentrations)		0
Hymenolepis				
nana	0	+	+	0
citelli	+	+	+	+
diminuta	+	+	+	+
Oochoristica				
symmetrica	(lethal)	+	+	0

The differential effects of certain gastrointestinal factors on excystment of tapeworm larvae *in vitro*. Data from Malkani (1933), deWaele (1934), Read (1955), Rothman (1958). 0 = not required or without effect; + = required or effective; ++ = marked effect.

There are other evidences that in some cases physiological incompatibility may be restricted to a particular step in the life cycle. *Echinococcus granulosus* and *E. multilocularis* furnish pertinent examples. The former species develops as the well known hydatid cyst in large mammals such as sheep, pig, and man but is apparently incapable of developing in rodents. On the other hand, *E. multilocularis* develops in certain rodents when these animals ingest the eggs of the worm. Schiller and Read (1958) have recently shown that there is no apparent lack of ability of rodent tissues to support the development of *E. granulosus*. Living cysts of *E. granulosus* removed from man at surgery were broken up in a blender for 10 seconds. This material was injected intraperitoneally in hamsters, mice, rats, and a monkey. Growth occurred in all these hosts. Parentheti-

cally, it may be emphasized that this form has not developed in a multilocular pattern in these animals. These observations strongly support the hypothesis that the failure of *E. granulosus* to infect rodents when these animals ingest eggs must be due to a failure of the early larvae to invade the tissues. It would be extremely interesting to study the comparative physiology of onchosphere liberation in these two species, since this may be the determining factor in intermediate host distribution.

These observations on *Echinococcus* are reminiscent of the studies of *Taenia pisiformis* reported by Leonard and Leonard (1941). These investigators found that if the onchospheres of *T. pisiformis* were inoculated into the mesenteric vein, there was no significant difference in the number of young developing larval worms in resistant and control rabbits. Leonard and Leonard concluded that the intestinal mucosa serves as an important barrier in preventing the onchospheres from reaching the vascular system in resistant hosts. However, we are dealing with qualitatively different phenomena in the cited examples of *Echinococcus* and *Taenia.* In one case, we are probably dealing with an insusceptibility and in the other with an acquired resistance. The basis for the insusceptibility may be an absence of stimuli necessary for onchosphere hatching and/or penetration. That of the acquired resistance may be the presence of inhibitory substances or cellular reactions which interfere with the hatching and/or penetration of the onchosphere. These possibilities should merit experimental study.

My colleague, Dr. Laurie, (1958) has studied the capacity of a variety of metabolites to induce encystment of the cercaria of the trematode, *Himasthla quissetensis.* In its life cycle this species encysts in certain bivalve molluscs (Stunkard, 1938). Laurie observed that some specificity was evident in the induction of encystment by different chemical substances. Encystment was induced by the following compounds, in order of relative activity: lysine, glucose, cellobiose, alpha-methylglucoside, galactose, gluconic acid, maltose, glutamic acid, mannose, glucosamine, xylose, arginine, and 3-0-methylglucose. Glucose and glutamic acid together produced an additive effect. A casein hydrolysate was more effective than any of the single compounds tested and the activity of the hydrolysate was enhanced by the addition of glucose. On the other hand, the following compounds did not induce encystment, during periods of exposure up to 24 hours: fructose, sorbose, 2-deoxyglucose, arabinose, rhamnose, trehalose, sucrose, lactose, melibiose, raffinose, glucosyloxime, dulcitol, sorbitol, i-inositol, mannitol, glucuronic acid, mucic acid, glycerophosphate, succinate, citrate, malonate, lactate, beta-alanine, glycine, methionine sulfoxide, DL-threonine, L-tyrosine, yeast nucleic acid, acetamide, and urea. Significantly, beta-alanine was found to antagonize the

induction of encystment by lysine. These observations indicate that there may not only be some specificity in the capacity of intermediate host secretions to induce encystment of *Himasthla* cercaria but that the presence of a specific substance in such fluids may inhibit encystment even in the presence of stimulatory compounds. Investigation of this hypothesis seems immediately feasible.

Recent studies by Sommerville (1957) and Rogers and Sommerville (1957) on the physiology of exsheathment of trichostrongyle nematode larvae may prove to be of great importance in understanding insusceptibility of some hosts to certain nematode species. These workers have presented evidence that there is a chemical stimulation afforded by host secretions which triggers the secretion by the nematode of substances causing exsheathment. It was shown that the exsheathing stimulant in host secretions is dialysable (in part) and heat-labile, and that its activity is affected by hydrogen ion concentration and oxidation-reduction potential. Optima for the former physical factor differed with the nematode species tested. The data suggest that there are a sufficient number of interdependent variables involved to expect that exsheathment would not occur in all animals *at sites allowing the survival of the fourth stage.* This assumes significance above the speculative level in view of the observation of Madsen and Whitlock (personal communication) that, in sheep showing natural insusceptibility to *Haemonchus,* the number of larvae exsheathing is markedly less than in genetically susceptible sheep.

Alteration of Susceptibility by External Factors

There is literature dealing with the relationships between nutrition and parasitism. These studies have for the most part been concerned with medical and veterinary problems and the emphasis has most often been on the aggravation of malnutrition by parasitism or vice versa. However, it has been pointed out that state of nutrition may interfere with the establishment of parasitism (Chandler, 1957). A few instances may be cited: Some years ago, Reiner and Paton (1932) showed that when rats are fed on diets deficient in B-complex vitamins, the growth of *Trypanosoma equiperdum* is inhibited. As far as I can ascertain, no further studies of this have been made. Seeler and Ott (1944) showed that the feeding of a riboflavin-deficient diet interfered with the multiplication of *Plasmodium lophurae.* There has been a considerable amount of recent interest in the finding that a low intake of para-aminobenzoic acid by the host suppresses the growth of *Plasmodium knowlesi* (Geiman and McKee, McKee and Geiman, 1948) and *P. berghei* (Maegraith, *et al.* 1952; Hawking, 1954; Fulton and Spooner, 1955; and others). The latter parasite seems to be adversely affected by a variety of environmental alterations,

including the dietary deficiencies (see above), pituitary somatotrophic hormone (Galliard, *et al.*, 1954), or the presence of another infectious agent (Adler, 1954). In some of these cases, additional study is required to determine whether there is an alteration of susceptibility or resistance or both.

Reducing agents in the host diet have been found to alter the course of certain parasitisms. McKee and Geiman (1946) showed that a deficiency of dietary ascorbic acid reduces the numbers of *Plasmodium knowlesi* in monkeys although, *in vitro*, lack of ascorbic acid in the medium had no appreciable effect on parasite multiplication. Godfrey (1957a) showed that the addition of cod liver oil to a stock diet suppressed the growth of *Babesia rodhaini*. Addition of vitamins A and D did not cause suppression of the piroplasm, but the unsaturated fatty acid fraction of cod liver oil was a highly effective suppressant. The cod-liver-oil suppression was reversed by adding ascorbic acid, vitamin E, or tetraethyl thiuram disulfide to the host diet. Addition of cod liver oil to the host diet was also shown to suppress the growth of *Plasmodium berghei*. This suppression was also antagonized by the addition of antioxidants to the diet (Godfrey, 1957b). In explanation of his observations, Godfrey suggested that the unsaturated fatty acids of cod liver oil are oxidized in the host's tissues to peroxides which inhibit development of the parasite. The reversal of the effect by antioxidant compounds strongly supports Godfrey's hypothesis. Greenberg *et al.* (1954) reported that feeding of maize oil, butter fat, and the fatty fraction from dried milk suppressed *Plasmodium gallinaceum* infection in chicks. These substances do not have similar effects when fed to mice with *P. berghei* infections (Godfrey, 1957b). Keppie (1953) reported that feeding mice diets composed of starch, salts, dried yeast, margarine, cod liver oil, and varying quantities of casein to mice inoculated with *Trypanosoma congolense* resulted in infections of low intensity or no infection at all. Godfrey has carried out further studies on effects of diets containing cod liver oil on infections with *Trypanosoma* spp., but these studies have not yet been published (Godfrey, 1957a).

The apparently contradictory nature of evidence for and against an ascorbic acid requirement by trypanosomes (reviewed by Lwoff, 1951) may be due to indirect effects of a similar type. (Lwoff has not discussed this evidence objectively. For example, the statement, ". . . it is well known that Trypanosomidae are strict aerobes . . ." is not justified by published information, and Mr. R. Zeledon, in our own laboratory, is currently growing several species of hemoflagellates in anaerobic culture. While it is true that some hemoflagellates are obligate aerobes, some are not.)

Whitlock (1949) reported that, when sheep were given supplementary

food, there was an apparently increased resistance to trichostrongylids in the small intestine but an apparently decreased resistance to *Haemonchus*. Experimental animals on a nutritionally optimum diet had more anemia and more *Haemonchus* (three times as many) than pasture-fed animals. This is probably a case in which a marked increase in susceptibility overrides a lesser increase in resistance. Since *Haemonchus* produces more pathology than the other sheep trichostrongylids, the practical importance of these observations is obvious.

Beaver (1937) probably observed a susceptibility alteration when he found that pigeons become refractory to infection with *Echinostoma revolutum* when maintained on a laboratory diet of yellow corn, with some wheat, oats, and rice added. Many workers have had the experience of bringing a wild animal into the laboratory and sorrowfully watching it lose its parasites. In some cases this may be due to the unavoidable alterations of host nutrition, with concomitant alterations of susceptibility.

During the past two years, my colleagues and I have published a series of studies on the role of carbohydrates in the biology of cestodes. These and related studies of others have been summarized and appropriate conclusions drawn (Read, 1958). It was concluded that (1) tapeworms require carbohydrates for growth, maintenance of size, and reproduction; (2) the carbohydrate requirements of the worms are satisfied by utilization of carbohydrate ingested by the host before such material has been absorbed by the host; (3) most tapeworms are capable of utilizing a few monosaccharide sugars but are incapable of utilizing di- or polysaccharides; (4) alteration of the quality of carbohydrate in the host diet results in reduction or increase in worm size, growth rate, egg production, and, in some instances, initial establishment of tapeworms; (5) the effects of host dietary carbohydrates of differing quality on tapeworms may be explained in terms of the physiology of the gut. It is plain that, in nature, selection of food of particular quality in carbohydrate content may play a part in determining whether and to what extent a parasitism of a particular worm species and a particular host species will be maintained.

The plant pathologists have amassed a considerable quantity of information on the effects of altering external environmental factors on the course of parasitisms. Such factors are potassium, nitrogen, or phosphorus content of the soil, pH, temperature, and humidity. Alterations of one or all of these factors may determine whether there will be parasitism of a plant in the presence of bacteria, fungi, nematodes, or viruses (Walker, 1957). It seems likely that in most cases these involve alterations of susceptibility. Incidentally, resistance also occurs in plants. For

example, the cellular reaction of certain strains of wheat to the fungus *Puccinia,* results in the death of the parasite (Allen, 1923). However, antibody formation has not been demonstrated in plants, and the information on cellular responses has been gained primarily in connection with studies of pathology *per se.*

There are instances which clearly suggest that different behavior patterns of sympatric host species may be involved in determining the distribution of parasites. For example, Read and Millemann (1953) found *Oochoristica deserti* Millemann in *Dipodomys merriami merriami* and *Citellus leucurus* but not in *D. panamintinus mohavensis* trapped in the same locality. Millemann (1953, 1955) corroborated this with more extensive trapping data and showed that although *D. panamintinus mohavensis* does not harbor *Oochoristica* in nature it is readily infected with this tapeworm in the laboratory. This might represent an example of host insusceptibility which is behavioral. There are alternative behavioral hypotheses: *D. panamintinus mohavensis* may eschew the eating of the intermediate host or this mammal may choose items in its natural diet which render its gut unfit as a habitat for *Oochoristica.* Another example which may be cited involves studies which are still in progress in our laboratory. These studies have been carried out in collaboration with Drs. E. L. Schiller, D. E. Davis, and V. Flyger. Schiller (1958) showed that the gray squirrel, *Sciurus carolinensis,* was readily infected with *Hymenolepis nana* in the laboratory. These animals were fed on dog biscuits. As a matter of fact, *H. nana* develops more rapidly to a larger size, lives longer and produces more eggs in *Sciurus* than in its "natural host," the mouse. However, *H. nana* has never been reported as a parasite of wild-caught *Sciurus.*

It seemed feasible to introduce this parasite into a previously studied, isolated, wild population of gray squirrels and determine the course of the infection. This was done, 10% of the population being infected in the field. Recaptures over a period of several months showed that the infection did not spread in this population and, as a matter of record, *did not develop significantly in those hosts initially infected.* This strongly suggested that the feeding habits of squirrels may result in an intestinal environment which is not at all suitable for *H. nana.* This has been tested in the laboratory by furnishing infected captive squirrels with various foods which they have been observed to utilize in nature. The results support the hypothesis that on certain diets the squirrel is insusceptible. This work will be reported in detail elsewhere. This seems to furnish an excellent example of a species which is insusceptible to a particular parasite by virtue of the foods which are chosen under natural conditions. I feel reasonably confident that if a wild population of *Sciurus* were fed

on dog biscuits, *H. nana* could readily establish itself in this host under "natural" conditions. We are attempting to test this experimentally by furnishing a wild population with dog biscuits.

In considering behavioral aspects of susceptibility, the behavior of the parasite should receive attention. The relationships of behavior to specificity in symbiosis have been reviewed recently by Davenport (1955). The conclusion seems inescapable that chemotropic responses of symbiotes to their hosts are widespread and, in some cases, sufficiently specific to account for the host distribution observed in nature. Painstaking comparative study of the chemotropic responses of the free-living stages of some parasites might shed light on certain problems of host and geographical distribution. For example, there is evidence that the miracidium of *Schistosoma japonicum* shows chemotactic responses to a specific snail and its mucus tract (Faust and Meleney, 1924). On the other hand, *Fasciola hepatica* miracidia show highly non-specific chemotropic responses (Neuhaus, 1953). Are these properties merely coincidental with the fact that *Fasciola hepatica* has an essentially cosmopolitan, though spotty, distribution, whereas *Schistosoma japonica* is restricted in its distribution to certain areas of the Orient? The specific behavioral responses of parasites to chemical stimuli derived from potential hosts have not received enough consideration from parasitologists.

Sociopsychological Stresses and Resistance

In spite of my stated effort to delete antigens and antibodies from this discussion, some mention must be made of an aspect of resistance which may involve behavior and certain concomitant effects on the capacity of host tissues to interfere responsively with establishment and maintenance of parasitism. There is a considerable body of evidence that, in the mammal, stresses of various types stimulate the release of adrenocorticotrophic hormone from the anterior pituitary via pathways involving the higher brain centers and the hypothalamus, with an increase in production of adrenal glucocorticoids (Harris, 1956). There is evidence that the injection of adrenal corticoids decreases the inflammatory responses of vertebrate tissues to foreign proteins or infectious agents. It has been shown that this is accompanied by an apparent loss of resistance to a number of parasitic organisms, such as viruses (Schwartzmann, 1950), bacteria (Berlin *et al.*, 1952), yeasts (Seligmann, 1953), rickettsiae (Whitmire and Downs, 1954), and helminths (Stoner and Godwin, 1953, 1954; Weinstein, 1953, 1955; Coker, 1955, 1956 a, b, c). As might be anticipated, stresses induced a suppression of inflammatory responses in the vertebrate, and resistance to foreign biochemical stimuli in general are depressed (Selye, 1952).

During the past 6 years evidence has accumulated in support of the concept that population dynamics of mammals, specifically fluctuations in numbers, are governed to a considerable extent by sociopsychological stresses. The evidence has recently been reviewed by Christian (1958) and will not be reviewed in detail here. The pertinent evidence indicates that, with increasing density of population, resulting psychogenic stresses produce increased activity of the pituitary-adrenocortical system. This is not referable to lack of food, water, nesting space, nor materials for nest-building but is attributable to social competition which can be defined in "units of social pressure." Thus, the stress of increasing contact with individuals of the same species results in an increase in the activity of the adrenal cortex, as well as inducing other effects on gonadal physiology. This leads us to offer the hypothesis that sociopsychological stresses, such as crowding, may produce changes in the physiology of the mammal which result in increased adrenal activity and a lowering of the tissue responses which interfere with the establishment or extent of parasitism. To my knowledge, not a single experimental test of this probability has been published. Dr. D. E. Davis and I are currently carrying out experimental work to test the hypothesis which was arrived at through the collaborative thinking of Dr. J. J. Christian, Dr. Davis, and, to a minor extent, myself. This hypothesis furnishes diverse ramifications. We may see that the physiological opportunities for establishment of parasitism would involve a variable which is independent of the variable of ecological opportunity. We may visualize the possibility that the establishment of parasitism involving new host-parasite combinations may be a function of population density, exclusive of the probabilities of transmission at varying densities. It is easy to see why this may have been overlooked previously in wild populations. Similar conditions representing social stresses may have direct effects on the course of parasitism; the obvious example is the enhancement of contagion with increasing population densities.

Further, it is important to point out that other stresses may be important in this connection. Malnutrition, for example, has been shown to serve as a stress which increases adrenocortical activity (Selye, 1946). One wonders if the many forms of malnutrition which have been shown to alter the character of parasitisms in laboratory animals and are thought to produce "disoperations" in parasitisms of human populations (Chandler, 1957) may not produce some of their effects through pituitary-adrenocortical stimulation, with resultant depression of resistance responses, rather than through direct depletion of the antibody-synthesizing systems.

There are possibilities for practically unlimited speculations and questions on the roles of population size, position of the individual in the social hierarchy, populations of other species, and other features of the environment acting as stressors through the host's endocrine system to modify parasitisms. I shall not indulge in further speculation on the point, but it would seem that this may be a very fertile field for investigation.

In dealing with problems involving alterations of innate susceptibility of hosts to disease agents the term "mutation" usually enters the discussion. In the case of vertebrates we may reasonably expect that alterations of genetic material will underlie alterations of innate susceptibility. This will certainly be true in those instances involving biochemical susceptibility whether this be due to the presence of an inhibitory substance or absence of a required substance. It may be pointed out that this will also be true of behavioral susceptibility. The genetic basis of vertebrate behavior has been pointed out by various authors (Tinbergen, 1953) and is frequently considered to be an important mechanism of isolation (Huxley, 1942). Thus, acquisition of behavioral insusceptibility might be expected to require alteration of genetic makeup. A most interesting exception to this is man. Non-genetic alterations of human behavior having the same significance for the species that genetic mutations may have for other vertebrates are readily recognizable. Some authors (e.g. Linton, 1955), have referred to the use of fire, the wheel, etc., as "mutations." In this same sense, the use of DDT, antibiotics, sewage disposal, and such may be regarded as alterations of behavioral resistance of the species without concomitant genetic alteration. It is interesting to point out that in man non-genetic alterations of behavioral susceptibility lead to alterations of age composition of populations and may conceivably lead to alterations of genetic composition, putting the shoe on the other foot. The role of non-genetic behavioral mutations in the isolation of human populations has interesting implications well outside the scope of the present discussion.

In summary, it may be said that the study of physiological and behavioral susceptibilities to animal parasites is in a primitive state. The foregoing discussion may contribute little to general understanding of the subject, but it is earnestly hoped that it may interest others in experimental consideration of the multitude of problems at hand. We may feel a bit like Dr. E. Racker's drunkard who lost the key to his door and searched for it under the street lamp where the light was more favorable for the search. Dr. Racker's friend found a key, but in his drunkenness he could not determine if it was his own. But what a fine time he had, sitting on his doorstep, dreaming of all the castles it might open.

REFERENCES

Adler, S. 1954. The behavior of *Plasmodium berghei* in the golden hamster *Mesocricetus auratus* infected with visceral leishmaniasis. *Trans. Roy. Soc. Trop. Med. Hyg.* **48**, 431-440.

Allen, R. F. 1923. Cytological studies of infection of Baart, Kanred, and Mindum wheats by *Puccinia graminis* forms III and XIX. *J. Agr. Res.* **26**, 571-604.

Beaver, P. B. 1937. Experimental studies on *Echinostoma revolutum* (Froelich) a fluke from birds and mammals. *U. Ill. Biol. Monog.* **15**, 1-96.

Beck, S. D. 1957. The European corn borer, *Pyrausta nubilalis* (Hübn.), and its principal host plant—VI. Host plant resistance to larval establishment. *J. Insect Physiol.* **1**, 158-177.

Berlin, B. S., Johnson, C., Hawk, W. D., and Lawrence, A. G. 1952. The occurrence of bacteremia and death in cortisone-treated mice. *J. Lab. Clin. Med.* **40**, 82-89.

Chandler, A. C. 1957. Interrelations between nutrition and infectious disease in the tropics. *Am. J. Trop. Med. Hyg.* **6**, 195-208.

Chitty, D. 1954. Tuberculosis among wild voles with a discussion of other pathological conditions among certain mammals and birds. *Ecology* **35**, 227-237.

Christian, J. J. 1958. A review of the endocrine responses in rats and mice to increasing population size including delayed effects on offspring. In *Am. Assoc. Adv. Sci. Symp. on Research of Sociological Significance.* In press.

Coker, C. M. 1955. Effects of cortisone on *Trichinella spiralis* infections in non-immunized mice. *J. Parasitol.* **41**, 498-504.

Coker, C. M. 1956. Cellular factors in acquired immunity to *Trichinella spiralis,* as indicated by cortisone treatment of mice. *J. Inf. Dis.* **98**, 185-197.

Coker, C. M. 1956. Some effects of cortisone in mice with acquired immunity to *Trichinella spiralis. J. Inf. Dis.* **98**, 39-44.

Coker, C. M. 1956. Effects of cortisone on cellular inflammation in the musculature of mice given one infection with *Trichinella spiralis. J. Parasitol.* **42**, 479-484.

DeWaele, A. 1934. Étude de la fonction biliare dans le phénomène de l'evagination chez les cysticerques des cestodes. *Ann. Parasitol.* **12**, 492-510.

Dubos, R. J. 1954. *Biochemical Determinants of Microbial Diseases.* Harvard U. Press, Cambridge. 152 pp.

Eisenbrandt, L. L., and Ackert, J. E. 1941. Effects of duodenal mucus of dogs and swine upon the viability of *Ascaridia lineata* in vitro. *J. Parasitol.* **27** (Suppl.), 36.

Faust, E. C., and Meleney, H. E. 1924. Studies on schistosomiasis japonica. *Am. J. Hyg. Monogr.* **3**, 339 pp.

Frick, L. P., and Ackert, J. E. 1948. Further studies on duodenal mucus as a factor in age resistance of chickens to parasitism. *J. Parasitol.* **34**, 192-206.

Fulton, J. D., and Spooner, D. F. 1955. The biochemistry and nutrition of *Plasmodium berghei. Indian J. Malariol.* **9**, 161-176.

Galliard, H., Lapierre, J., and Murard, J. 1954. Inhibition de l'infection à *Plasmodium berghei* chez la souris et le rat par l'hormone hypophysaire de croissance (S.T.H.). *Ann. Parasitol. Hum. Comp.* **29**, 167-178.

Geiman, Q. M., and McKee, R. W. 1948. Malarial parasites and their mode of life. *Sci. Monthly* **67**, 217.

Godfrey, D. G. 1957a. The influence of dietary cod liver oil and vitamin E upon *Babesia rodhaini* in mice. *Exp. Parasitol.* **6**, 465-485.

Godfrey, D. G. 1957b. Antiparasitic action of dietary cod liver oil upon *Plasmodium berghei* and its reversal by vitamin "E." *Exp. Parasitol.* **6**, 555-564.

Greenberg, J., Taylor, D. J., and Trembley, H. L. 1954. The effect of milk diets on the course of sporozoite-induced *Plasmodium gallinaceum* infections in chicks. *Am. J. Hyg.* **60**, 99-105.

Harris, G. W. 1956. Hypothalamic control of the anterior lobe of the hypophysis. In *Hypothalamic-hypophyseal interrelationships,* Ed. W. S. Fields, R. Guillemin, and C. A. Carton. Chas. C Thomas, Springfield.

Hawking, F. 1954. Milk, p-aminobenzoate, and malaria of rats and monkeys. *Brit. Med. J.* **1**, 425-429.

Huxley, J. 1942. *Evolution the Modern Synthesis.* Harper, N.Y. 645 pp.

Johann, H., and Dickson, A. D. 1945. A soluble substance in cornstalks that retards growth of *Diplodia zeae* in culture. *J. Agr. Res.* **71**, 89-110.

Keppie, A. N. 1953. Modified course of *Trypanosoma congolense* infection in mice given diets with milk casein. *Brit. Med. J.* **2**, 853-867.

Larsh, J. E., Jr. 1947. The relationship in mice of intestinal emptying time and natural resistance to *Hymenolepis. J. Parasitol.* **33**, 79-84.

Larsh, J. E., Gilchrist, H. B., and Greenberg, B. G. 1952. A study of the distribution and longevity of adult *Trichinella spiralis* in immunized and non-immunized mice. *J. Elisha Mitchell Sci. Soc.* **68**, 1-11.

Larsh, J. E., Jr., Race, G. J., and Jeffries, W. B. 1956. The association in young mice of intestinal inflammation and the loss of adult worms following an initial infection with *Trichinella spiralis. J. Inf. Dis.* **99**, 63-71.

Laurie, J. S. 1958. Induced *in vitro* encystment of the cercaria of *Himasthla quissetensis.* Unpublished ms.

Leonard, A. B., and Leonard, A. E. 1941. The intestinal phase of the resistance of rabbits to the larvae of *Taenia pisiformis. J. Parasitol.* **27**, 375-378.

Link, K. P., and Walker, J. C. 1933. The isolation of catechol from pigmented onion scales and its significance in relation to disease resistance in onions. *J. Biol. Chem.* **100**, 379-383.

Linton, Ralph. 1955. *The Tree of Culture.* Knopf, N.Y. 692 pp.

Lwoff, M. 1951. The nutrition of parasitic flagellates (Trypanosomidae, Trichomonadinae). In *Biochemistry and physiology of protozoa* I. Ed. A. Lwoff. Acad. Press, N.Y. pp. 129-176.

Maegraith, B. G., Deegan, T., and Jones, E. S. 1952. Suppression of malaria *(P. berghei)* by milk. *Brit. Med. J.* **2**, 1382-1384; 1405.

Malkani, P. G. 1933. A rapid method of evaginating the scolices in parasitic cysts. *Indian Vet. J.* **10**, 122-124.

McKee, R. W., and Geiman, Q. M. 1946. Studies on malarial parasites. V. Effects of ascorbic acid on malaria in monkeys. *Proc. Soc. Exp. Biol. Med.* **63**, 313-315.

McKee, R. W., and Geiman, Q. M. 1948. Methionine in the growth of the malarial parasite, *Plasmodium knowlesi. Fed. Proc.* **7**, 172.

Michajlow, W. 1951. Stadialnosc rozwoju niektorych tasiemcow (Cestoda). (Uderzajca analogia biologiczna). *Ann. Universit. Mariae Curie-Sklowdowska* **6**, 77-147.

Millemann, R. E. 1953. Studies on the biology of the cestode, *Oochoristica deserti* n. sp. Ph.D. Dissertation, Univ. Calif., Los Angeles.

Millemann, R. E. 1955. Studies on the life-history and biology of *Oochoristica deserti* n. sp. (Cestoda: Linstowiidae) from desert rodents. *J. Parasitol.* **41**, 424-440.

Neuhaus, W. 1953. Über den chemischen Sinn der Miracidien von *Fasciola hepatica. Zeitschr. Parasitenk.* **15**, 476-490.

Prévost, B. 1807. Mémoire sur la cause immédiate de la carie ou charbon des blés, et de plusieurs autres maladies des plantes, et sur les préservatifs de la carie. Paris, 1807. Engl. transl. by G. W. Keitt in *Phytopath. Classics* **6** (1939).

Racker, E. 1954. Metabolism of infected cells. In *Cellular Metabolism and Infections.* Ed. E. Racker. Academic Press, N.Y. pp. 138-148.

Read, C. P. 1955. Intestinal physiology and the host-parasite relationship. In *Some Physiological Aspects and Consequences of Parasitism.* Ed. W. H. Cole, Rutgers Univ. Press. pp. 27-43.

Read, C. P. 1958. The role of carbohydrates in the biology of cestodes. VIII. Some conclusions and hypotheses. *Exp. Parasitol.* In press.

Read, C. P., Douglas, L. T., and Simmons, J. E. 1958. Urea and osmotic properties of tapeworms from elasmobranchs. *Exp. Parasitol.* In press.

Read, C. P., and Millemann, R. E. 1953. Helminth parasites in kangaroo rats. *Univ. Calif. Publ. Zool.* **59**, 61-80.

Read, C. P., and Voge, M. 1954. The size attained by *Hymenolepis diminuta* in different host species. *J. Parasitol.* **40,** 88-89.

Rogers, W. P., and Sommerville, R. I. 1957. Physiology of exsheathment in nematodes and its relation to parasitism. *Nature* **179,** 619-621.

Rothman, A. H. 1958a. Sc.D. Dissertation, Johns Hopkins University.

Rothman, A. H. 1958b. The role of bile salts in the biology of tapeworms. I. Effects of bile salts on the metabolism of *Hymenolepis diminuta* and *Oochoristica symmetrica. Exp. Parasitol.* In press.

Rothman, A. H., and Read, C. P. 1957. Effects of bile salts on tapeworm metabolism. *Anat. Rec.* **128,** 611.

Schaal, L. A., and Johnson, G. 1955. The inhibitory effect of phenolic compounds on the growth of *Streptomyces scabies* as related to the mechanism of scab resistance. *Phytopathol.* **45,** 626-628.

Schiller, E. L. 1958. Sc.D. Dissertation, Johns Hopkins University.

Schiller, E. L., and Read, C. P. 1958. Experimental studies on *Echinococcus granulosus.* Ms. in preparation.

Schneider, T. A. 1951. Nutrition and resistance—susceptibility to infection. *Am. J. Trop. Med.* **31,** 174-182.

Schwartzmann, G. 1950. Enhancing effect of cortisone upon poliomyelitis infection (strain MEF1) in hamsters and mice. *Proc. Soc. Exp. Biol & Med.* **75,**835-838.

Seeler, A. O., and Ott, W. H. 1944. Effect of riboflavin deficiency on the course of *Plasmodium* infection in chicks. *J. Inf. Dis.* **75,** 175.

Seligmann, E. 1953. Virulence enhancement of *Candida albicans* by antibiotics and cortisone. *Proc. Soc. Exp. Biol. & Med.* **83,** 778-781.

Selye, H. 1946. The general adaptation syndrome and the diseases of adaptation. *J. Clin. Endocrinol.* **6,** 117-230.

Selye, H. 1951. First annual report on stress. Acta, Inc., Montreal.

Sommerville, R. I. 1957. The exsheathing mechanism of nematode infective larvae. *Exp. Parasitol.* **6,** 18-30.

Stoner, R. D., and Godwin, J. T. 1953. The effects of ACTH and cortisone upon susceptibility to trichinosis in mice. *Am. J. Pathol.* **29,** 943-950.

Stoner, R. D., and Godwin, J. T. 1954. The effects of adrenocorticotrophic hormone and cortisone upon acquired immunity to trichinosis in mice. *Am. J. Pathol.* **30,** 913-918.

Stunkard, H. W. 1938. The morphology and life cycle of the trematode, *Himasthla quissetensis* (Miller and Northrup, 1926). *Biol. Bull.* **75,** 145-164.

Tinbergen, N. 1953. *Social Behavior in Animals.* Wiley, N.Y. 150 pp.

Virtanen, A. I., and Hietala, P. K. 1955. 2(3)-Benzoxazolinone, an antifusarium factor in rye seedlings. *Acta Chem. Scand.* **9,** 1543-1544.

Virtanen, A. I., Hietala, P. K., and Wahlroos, O. 1956. An antifungal factor in maize and wheat plants. *Swom. Kemist.* **29 B,** 143-144.

Walker, J. C. 1923. Disease resistance to onion smudge. *J. Agr. Res.* **24,** 1019-1039.

Walker, J. C. 1957. *Plant Pathology.* McGraw-Hill, N.Y. 707 pp.

Weinstein, P. P. 1953. The effect of cortisone on the development of the immune response in the white rat to *Nippostrongylus muris. J. Parasitol.* **39** (Suppl.), 35.

Weinstein, P. P. 1955. The effect of cortisone on the immune response of the white rat to *Nippostrongylus muris. Am. J. Trop. Med. Hyg.* **4,** 61-74.

Whitlock, J. H. 1949. The relationship of nutrition to the development of the trichostrongylidoses. *Cornell Vet.* **39,** 146-182.

Whitmire, C. E., and Downs, C. M. 1954. Effect of cortisone on experimental murine typhus in mice. *Science* **119,** 468-469.